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Knockdown of glycine decarboxylase complex alters photorespiratory carbon isotope fractionation in *Oryza sativa* leaves

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Abstract

The influence of reduced glycine decarboxylase complex (GDC) activity on leaf atmosphere CO₂ and ¹³CO₂ exchange was tested in transgenic *Oryza sativa* with the GDC H-subunit knocked down in leaf mesophyll cells. Leaf measurements on transgenic *gdch* knockdown and wild-type plants were carried out in the light under photorespiratory and low photorespiratory conditions (i.e. 18.4 kPa and 1.84 kPa atmospheric O₂ partial pressure, respectively), and in the dark. Under approximately current ambient O₂ partial pressure (18.4 kPa pO₂), the *gdch* knockdown plants showed an expected photorespiratory-deficient phenotype, with lower leaf net CO₂ assimilation rates (*A*) than the wild-type. Additionally, under these conditions, the *gdch* knockdown plants had greater leaf net discrimination against ¹³CO₂ (Δ_o) than the wild-type. This difference in Δ_o was in part due to lower ¹³C photorespiratory fractionation (*f*) ascribed to alternative decarboxylation of photorespiratory intermediates. Furthermore, the leaf dark respiration rate (*R_d*) was enhanced and the ¹³CO₂ composition of respired CO₂ ($\delta^{13}\text{C}_{\text{Rd}}$) showed a tendency to be more depleted in the *gdch* knockdown plants. These changes in *R_d* and $\delta^{13}\text{C}_{\text{Rd}}$ were due to the amount and carbon isotopic composition of substrates available for dark respiration. These results demonstrate that impairment of the photorespiratory pathway affects leaf ¹³CO₂ exchange, particularly the ¹³C decarboxylation fractionation associated with photorespiration.

Keywords: ¹³C discrimination, C₄ photosynthesis, CO₂ exchange, GDC knockdown, leaf dark respiration, photorespiration, rice.

Introduction

In C₃ plants, Rubisco operates in the leaf mesophyll cells, where CO₂ and O₂ compete to react with ribulose-1,5-bisphosphate (RuBP). The carboxylation of RuBP results in the formation

of two molecules of 3-phosphoglycerate (3-PGA) that are integrated into the Calvin–Benson cycle. Alternatively, the oxygenation of RuBP produces one molecule of 3-PGA and one

2-phosphoglycolate (2-PG). The 2-PG is primarily recycled via photorespiration through a complex and energy-consuming set of reactions, which spans the chloroplasts, cytosol, peroxisomes, and mitochondria (Bauwe *et al.*, 2010; Betti *et al.*, 2016). By scavenging 2-PG, photorespiration removes a strong inhibitor of enzymes in photosynthetic carbon metabolism (Anderson, 1971; Kelly and Latzko, 1976; Peterhansel *et al.*, 2013b; Walker *et al.*, 2016) and recovers up to one molecule of 3-PGA for every two molecules of 2-PG. Nevertheless, a minimum of one out of four 2-PG carbon atoms is released as CO₂ by the glycine decarboxylase complex (GDC) and can be lost by the plant (Bauwe, 2018).

The GDC is an atypical mitochondrial four-protein system, comprised of three enzymes (P-, T-, and L-protein) and the H-protein, which is a small lipoylated protein (Somerville and Ogren, 1982; Douce *et al.*, 2001; Bauwe, 2018). GDC plays a critical role in the photorespiratory cycle by catalyzing the conversion of two molecules of glycine into serine and one molecule of CO₂ and NH₃ (Somerville, 2001; Maurino and Peterhansel, 2010). However, in the absence of the H component, the GDC cannot oxidize glycine (Douce *et al.*, 2001; Parys and Jastrzębski, 2008), which can accumulate. In C₃ plants, the impaired activity of the H-subunit leads to a knock-down (KD) of GDC activity and a photorespiratory phenotype (Ewald *et al.*, 2007). Plants with reduced GDC activity typically have lower rates of leaf photosynthesis, a depletion of Calvin cycle metabolites, an impairment of photorespiratory nitrogen re-assimilation, and the accumulation of photorespiratory metabolites (e.g. glycine) under current ambient CO₂ and O₂ partial pressures (Wingler *et al.*, 2000; Timm and Bauwe, 2013; Lin *et al.*, 2016).

This buildup of leaf photorespiratory metabolites can have a negative feedback effect on Calvin cycle activity. For example, glyoxylate produced by glycolate oxidation negatively impacts on the activation state of Rubisco (Wingler *et al.*, 1999; Peterhansel *et al.*, 2010). Additionally, disruption of the photorespiratory pathway may lead to alternative decarboxylation reactions of accumulated pools of photorespiratory intermediates, such as glyoxylate and hydroxypyruvate in the peroxisomes (Wingler *et al.*, 1999, 2000; Tcherkez, 2006; Peterhansel *et al.*, 2010), and an increase in the ratio of moles of photorespiratory CO₂ released per mole of O₂ reacting with RuBP (α ; Cousins *et al.*, 2008, 2011; Walker and Cousins, 2013; Timm *et al.*, 2018). Furthermore, the accumulation of photorespiratory intermediates could also affect the rates of leaf CO₂ evolved in the dark (R_d , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and the ¹³C composition of R_d ($\delta^{13}\text{C}_{R_d}$, ‰) (Ghashghaie *et al.*, 2003; Tcherkez *et al.*, 2003).

The multiple leaf metabolic reactions simultaneously consuming and releasing CO₂ in the light make it difficult to determine how changes in photorespiration affect rates of leaf net CO₂ assimilation (A), mesophyll CO₂ conductance (g_m), refixation of (photo)respired CO₂, and mitochondrial non-photorespiratory respiration rates (R_L). However, photosynthesizing leaves discriminate against ¹³C during CO₂ diffusion from the atmosphere to the chloroplast stroma (through both the air and liquid phases), and during carboxylation, photorespiration, and mitochondrial non-photorespiratory respiration

processes, with a specific ¹³C fractionation for each diffusional or biochemical step (Evans *et al.*, 1986). The observed leaf net discrimination against ¹³C in the light (Δ_o , ‰) can be modeled with four ¹³C fractionation terms (‰): Δ_i , which accounts for the ¹³C discrimination during CO₂ diffusion from the atmosphere to the intercellular air space and for the Rubisco ¹³C fractionation ($\sim 29\text{‰}$, Ubierna and Farquhar, 2014; von Caemmerer *et al.*, 2014); Δ_{gm} , which accounts for the ¹³C discrimination during CO₂ diffusion in the liquid phase to chloroplast stroma and depends on the magnitude of g_m ; and Δ_f and Δ_e which are associated with photorespiration and mitochondrial non-photorespiratory respiration activity, respectively (von Caemmerer and Evans, 1991; Flexas *et al.*, 2008; Tazoe *et al.*, 2011; Evans and von Caemmerer, 2013). Δ_f is primarily attributed to the glycine-serine reaction catalyzed by GDC, which releases CO₂ depleted in ¹³C compared with substrate and tends to decrease Δ_o (Farquhar *et al.*, 1982; Ghashghaie *et al.*, 2003; Lanigan *et al.*, 2008). In contrast, Δ_e may increase or decrease Δ_o in relation to the difference between ¹³C composition (‰) of CO₂ entering the leaf chamber during measurements and in the plant growth chamber (Gillon and Griffiths, 1997; Ghashghaie *et al.*, 2003).

The photorespiratory fractionation (f , ‰) estimated *in vivo* in multiple C₃ species ranges between 8‰ and 16.2‰ relative to photosynthetic products (Ghashghaie *et al.*, 2003; Evans and von Caemmerer, 2013), with 11‰ predicted from the theory (Tcherkez, 2006). However, under photorespiratory conditions, when Rubisco oxygenation exceeds the capacity of the photorespiratory recycling of 2-PG or in the presence of disruption of the photorespiratory pathway, Δ_f and f may vary due to changes in α associated with alternative decarboxylation of photorespiratory intermediates (Cousins *et al.*, 2008, 2011; Walker and Cousins, 2013). Alternative photorespiratory bypasses may occur in the chloroplasts (e.g. glyoxylate may be enzymatically reduced back to glycolate or further oxidized to CO₂, but with no RuBP regenerated; see Kebeish *et al.*, 2007), peroxisomes (non-enzymatic decarboxylation of glyoxylate to formate using H₂O₂ as oxidizing agent may lead to formation of serine; catalase may be also involved as reported in Wingler *et al.*, 1999), mitochondria (enzymatic oxidation of glycolate to glyoxylate with release of CO₂ and synthesis of glycine; see Niessen *et al.*, 2007), and cytosol (enzymatic reduction of hydroxypyruvate to glycerate; see Timm *et al.*, 2008).

The aim of the present study was to test how changes in carbon flux through the photorespiratory pathway influenced leaf CO₂ and ¹³CO₂ isotope exchange, both in the light and in the dark, in transgenic plants of *Oryza sativa* with the GDC H-subunit KD in mesophyll cells. Both *gdch*-KD and wild-type (WT) plants were grown under low photorespiratory conditions (atmospheric CO₂ partial pressure of 184.2 Pa) to minimize any pleiotropic effects. In the light, measurements of leaf-atmosphere CO₂ and stable carbon isotope exchange were performed under low photorespiratory and photorespiratory conditions (atmospheric O₂ partial pressure of 1.84 kPa or 18.4 kPa, respectively, and CO₂ partial pressure of 27.6 Pa). The disruption of the photorespiratory pathway in the *gdch*-KD plants was characterized by leaf photosynthetic traits, Δ_o , Δ_f , f , α , R_d , and $\delta^{13}\text{C}_{R_d}$, compared with the WT.

Materials and methods

Plant material

Generation of GDC-H knockdown transgenic rice lines

The generation and the characterization of three *Oryza sativa* *gdch*-KD transgenic lines, including *gdch*-38, was previously described by Lin *et al.* (2016). Line *gdch*-38 was selected for analysis in the present study since in Lin *et al.* (2016) it had shown a more consistent photorespiratory-deficient phenotype under different O₂:CO₂ growing and measuring conditions compared with the other two *gdch*-KD lines. Untransformed *O. sativa* cv. IR64 line A009 (WT) was used as negative control for comparison with the *gdch*-KD line.

Plant growth conditions

Two batches of 10 transgenic *gdch*-38 line (T₄ generation) and 10 WT plants of *O. sativa* cv. IR64 were grown consecutively in a controlled-environment growth chamber (G_{ch}; Bigfoot series, BioChambers Inc., Winnipeg, MB, Canada) at the School of Biological Sciences at Washington State University, Pullman, WA (USA). All plants were individually grown in 4 liter free drainage pots; soil, irrigation, and fertilization were as in Giuliani *et al.* (2013).

The daily photoperiod was 14 h, from 8.00 h to 22.00 h standard time. Light was provided by F54T5/841HO Fluorescent 4100 K and 40 W halogen incandescent bulbs (Philips) and was supplied in a bell-shaped pattern; that is, with increasing photosynthetic photon flux density (PPFD) during the first 2 h, a maximum PPFD of 600 μmol photons m⁻² s⁻¹ incident on the plant canopy for 10 h, and decreasing PPFD in the last 2 h. Air temperature (*t*_{air}) was set at 22 °C in the dark period; after switching on the light, *t*_{air} tracked the PPFD pattern; that is, it ramped during the first 2 h from 22 °C to 26 °C, then 26 °C for 10 h, and decreased to 22 °C in the last 2 h photoperiod. Air relative humidity was maintained at ~70%, corresponding to a maximum air vapor pressure deficit (VPD) of ~1.6 kPa. During the light period, the CO₂ partial pressure (*p*CO₂) in the G_{ch} atmosphere was elevated to 184.2 Pa (2000 μmol mol⁻¹). The ¹³C composition of the atmospheric CO₂ during the light period ($\delta^{13}\text{C}_{\text{Gch}}$) was -41.6‰ and -30.6‰ for the first and second batch of grown plants, respectively. The $\delta^{13}\text{C}_{\text{Gch}}$ was determined as described in Supplementary Methods S1 at JXB online, and was a proxy of the ¹³C composition of the CO₂ in the tank used (during the second plant growing cycle a new tank was needed and no tank with ¹³CO₂ composition comparable with the previous one was available).

Leaf biochemical analysis

Protein content

Protein immunoblot analysis was performed to determine the leaf abundance of GDC H-, P-, and T-subunits in fully expanded leaves of 4- to 5-week-old transgenic *gdch*-KD and WT plants. For each genotype, two separate protein extractions were performed, each one using the leaf tissue collected from two plants, according to Koteyeva *et al.* (2015). Protein concentration was determined for each extract with an RC DC protein quantification kit (Bio-Rad, Hercules, CA, USA) and 20 μg of protein per extract were separated by 10% (w/v) SDS-PAGE for the GDC P-subunit or 15% (w/v) for GDC H- and T-subunits. Proteins were then transferred to a nitrocellulose membrane and immunoblots (*n*=2 for both *gdch*-KD and WT) were performed according to Koteyeva *et al.* (2015) with primary antibodies for anti-*Pisum sativum* L. GDC H-, P-, and T-subunits (1:10 000) raised in rabbit (courtesy of Dr D. Oliver, Iowa State University). The L-subunit was not detected because antibodies were unavailable. The band intensities were quantified with ImageJ 1.37 software (NIH, USA).

Malate content

The leaf portions used for photosynthesis analysis in *gdch*-KD and WT plants (*n*=5) were sampled immediately after the leaf-atmosphere gas exchange measurements and frozen in liquid N₂. Malate content per unit leaf surface area (mmol malate m⁻²) was then determined with a

spectrophotometry-based assay as described by Hatch (1979), with modifications by Edwards *et al.* (1982).

Leaf physiological analysis

Coupled measurements of leaf-atmosphere CO₂, H₂O, and ¹³CO₂ exchange

Measurements were performed in Pullman, WA, USA with a mean atmospheric pressure of 92.1 kPa. Two LI-6400XT portable gas analyzers (LI-COR Biosciences, Lincoln, NE, USA; detecting ¹²CO₂) operating as open systems were coupled to a tunable diode laser absorption spectroscope, which detects ¹²CO₂ and ¹³CO₂ isotopologs (TDLAS model TGA200A, Campbell Scientific, Inc., Logan, UT, USA; Bowling *et al.*, 2003; Barbour *et al.*, 2007; Ubierna *et al.*, 2011; Stutz *et al.*, 2014; Sun *et al.*, 2014). Additional technical information on the system setup are available in Supplementary Methods S2.

For the leaf photosynthesis measurements, each LI-COR was equipped with a 2×3 cm leaf chamber (L_{ch}) assembled with an LED light source (6400-02B; LI-COR Biosciences). Alternatively, leaf dark respiration measurements were performed using an 8×10 cm custom-built L_{ch} having an adaxial glass window, and with a volume of ~100 cm³ (Barbour *et al.*, 2007, based on Sharkey *et al.*, 1985). The chamber had a hollowed stainless steel frame sealed with a closed-cell foam gasket and was connected to a circulating water bath for temperature control in the lumen. Before dark respiration measurements, the leaf portions included in the L_{ch} were exposed to the light, which was supplied by a LI-COR 6400-18 light source placed adjacent to the glass window.

Protocol for coupled measurements of leaf-atmosphere CO₂, H₂O, and ¹³CO₂ exchange

The mid to distal portions of two fully expanded leaves from the same stem on 4- to 5-week-old plants (*n*=4 for *gdch*-KD; *n*=5 for WT) grown under $\delta^{13}\text{C}_{\text{Gch}}$ of -41.6‰ were used for leaf photosynthetic measurements. The leaves were positioned to cover the 6 cm² L_{ch} section area. Measurements were taken from 10.00 h until 16.00 h standard time under an O₂ partial pressure (*p*O₂) of 18.4 kPa (approximately the current atmospheric *p*O₂) and 1.84 kPa, *p*CO₂ (*C*_i) of 27.6 Pa, and ¹³C composition of CO₂ (from a pressurized tank) entering the L_{ch} (δ_{in}) of -48.0‰. PPFD was set at 1500 μmol photons m⁻² s⁻¹, leaf temperature (*t*_{leaf}) at 25 °C, and leaf to air VPD was kept between 1.0 kPa and 1.5 kPa. The airflow rate through the LI-COR system was 300 μmol s⁻¹ (~0.48 l min⁻¹). In particular, a *C*_a below current ambient *p*CO₂ (which was ~37 Pa) was chosen to amplify, under a *p*O₂ of 18.4 kPa, the signals of the photorespiratory-deficient phenotype in the *gdch*-KD plants compared with the WT.

Under each experimental O₂ condition, leaf portions were acclimated for ~30 min and data were recorded for ~30–40 min. The rate of net CO₂ assimilation per unit (one side) leaf surface area (*A*, μmol CO₂ m⁻² s⁻¹), stomatal conductance to CO₂ diffusion (*g*_{sc}, μmol CO₂ m⁻² s⁻¹ Pa⁻¹), intercellular *p*CO₂ (*C*_i, Pa), and the ratio *C*_i/*C*_a were determined.

For leaf dark respiration measurements, *gdch*-KD and WT plants (*n*=4) grown at a $\delta^{13}\text{C}_{\text{Gch}}$ of both -41.6‰ and -30.6‰ were used. Two plants per day (one *gdch*-KD and one WT) were taken out of the G_{ch} at 9.30 h standard time and the mid to distal portions of 8–9 fully expanded leaves, similar to those used for the photosynthetic analysis, were enclosed in the custom-built L_{ch} to cover the section area of ~76 cm². Leaf portions were first exposed to a PPFD of 750 μmol photons m⁻² s⁻¹ for 20 min, 500 μmol photons m⁻² s⁻¹ for 15 min (at *t*_{leaf} of 25 °C), and 100 μmol photons m⁻² s⁻¹ for 5 min (at *t*_{leaf} of 30 °C). Measurements were taken under a *p*O₂ of 1.84 kPa or 18.4 kPa for plants grown at a $\delta^{13}\text{C}_{\text{Gch}}$ of -41.6‰ or -30.6‰, respectively. *C*_a was set at 35.0 Pa, and the airflow rate through the LI-COR was changed from 700 μmol s⁻¹ to 500 μmol s⁻¹, and from 500 μmol s⁻¹ to 350 μmol s⁻¹ tracking the decreasing PPFD. A CO₂ cartridge from a set of cartridges with $\delta^{13}\text{C}$ from -6.2‰ to -4.8‰ was used, one per day, as CO₂ source (the mean δ_{in} for all experimental conditions is shown in Supplementary Table S1). The different (higher) $\delta^{13}\text{C}_{\text{CO}_2}$ composition entering the L_{ch} with respect to the G_{ch} (-41.6‰) was chosen to have the leaf carbon assimilates

produced in the L_{ch} with dissimilar (higher) $\delta^{13}C$ signatures compared with those previously produced in the G_{ch} . After 40 min of leaf light exposure, darkness was imposed in the L_{ch} . Leaf CO_2 evolution was measured at a pO_2 of 18.4 kPa and t_{leaf} of 30 °C for 195 min to determine the dynamics of the dark respiration rate per unit (one side) of leaf surface area (R_d , $\mu mol CO_2 m^{-2} s^{-1}$) and corresponding $\delta^{13}C$ ($\delta^{13}C_{Rd}$, ‰). The t_{leaf} was set at 30 °C to enhance the precision of the dark measurements. Additionally, three plants ($n=3$) of the *gdch*-KD line and of the WT were taken out of the growth chamber at 12.00 h standard time 3 d after their use for measurements, and darkened at 25 °C for 24 h. Subsequently, leaf dark CO_2 evolution was measured at a t_{leaf} of 30 °C and a pO_2 of 18.4 kPa to determine $R_{d(24h)}$ ($\mu mol CO_2 m^{-2} s^{-1}$) and $\delta^{13}C_{Rd(24h)}$ (‰). The blade portions used for dark measurements on WT and *gdch*-KD plants were sampled and dried in a ventilated oven at 55 °C for 48 h to determine leaf dry mass per (one side) unit of leaf surface area (LMA, $g m^{-2}$).

For each *gdch*-KD and WT plant used for leaf photosynthesis measurements, the ^{13}C signature of leaf dry matter ($\delta^{13}C_{dm}$, ‰) and leaf total N content as a fraction (%) of dry matter were determined as described in [Supplementary Methods S3](#), and the leaf total N content per unit leaf surface area ($g m^{-2}$) was calculated. The descriptions, values, and units of abbreviations and symbols are listed in [Table 1](#).

Leaf net $^{13}CO_2$ discrimination in the light and mesophyll conductance to CO_2 diffusion

The observed leaf net discrimination against $^{13}CO_2$ in the light (Δ_o , ‰) was calculated by mass balance from the TDLAS measurements according to [Evans *et al.* \(1986\)](#). Under photorespiratory conditions (18.4 kPa pO_2), the $^{13}CO_2$ fractionation for photorespiration (f , ‰) in the *gdch*-KD plants was calculated based on [Evans and von Caemmerer \(2013\)](#). Briefly, the value of f was determined by modeling the leaf net discrimination against $^{13}CO_2$ (Δ_o) as a function of the ^{13}C discrimination fractions associated with CO_2 diffusion from the atmosphere to the intercellular air space and with carboxylation (Δ_i), with CO_2 diffusion in liquid phase to chloroplast stroma (Δ_{gm}), mitochondrial non-photorespiratory respiration (Δ_e), and photorespiration (Δ_p). The equation $\Delta_o = \Delta_i - \Delta_{gm} - \Delta_f - \Delta_e$ can be rearranged so that $\Delta_f = \Delta_i - \Delta_o - \Delta_{gm} - \Delta_e$ and f can be estimated by substituting Δ_f with $\Delta_f = \frac{1}{1-f} \left(f \frac{\Gamma^*}{C_a} \right)$ to get $\frac{1}{1-f} \left(f \frac{\Gamma^*}{C_a} \right) = \Delta_i - \Delta_o - \Delta_{gm} - \Delta_e$. An f value of 16.2‰ was taken from [Evans and von Caemmerer \(2013\)](#) and assumed for WT plants. The input parameters needed to calculate f include the leaf mitochondrial respiration rate in the light (R_L , $\mu mol CO_2 m^{-2} s^{-1}$), the CO_2 compensation point in the absence of mitochondrial non-photorespiratory respiration (Γ^* , $\mu mol mol^{-1}$), and mesophyll CO_2 conductance (g_m , $mol CO_2 m^{-2} s^{-1}$). Values of R_L at a t_{leaf} of 25 °C were modeled for both genotypes from the corresponding R_d at 30 °C after 3 h in the dark [$R_{d(3h)}$, $\mu mol CO_2 m^{-2} s^{-1}$] following leaf photosynthesis under atmospheric pO_2 of 18.4 kPa using the temperature response function in [Bernacchi *et al.* \(2001\)](#). The Γ^* was modeled based on [von Caemmerer \(2000\)](#), as described in [Supplementary Methods S4](#), and was significantly different ($P < 0.05$) between WT and *gdch*-KD plants, 45.0 ± 1.7 SE ($n=4$) $\mu mol mol^{-1}$ and 53.3 ± 0.6 SE ($n=3$) $\mu mol mol^{-1}$, respectively. Finally, g_m was estimated based on leaf-atmosphere CO_2 and $^{13}CO_2$ exchange data, according to [Evans and von Caemmerer \(2013\)](#). Specifically, ^{13}C -based g_m was calculated in the *gdch*-KD and WT plants at 1.84 kPa pO_2 , but only in WT plants under 18.4 kPa pO_2 , using an f value of 16.2‰. The ^{13}C -based g_m cannot be calculated in *gdch*-KD plants at 18.4 kPa pO_2 because g_m and f are not independent variables in the applied procedure. Therefore, at 18.4 kPa, the g_m values of *gdch*-KD plants were set the same as for the WT. This assumes that the ^{13}C -based g_m integrates the within-leaf resistances affecting CO_2 movement across the cell wall, plasma membrane, and the chloroplast membranes, and that this cumulative resistance does not differ between *gdch*-KD and WT plants. This assumption is supported by the fact that the ^{18}O -based g_m , which was determined by analysis of leaf-atmosphere ^{18}O exchange according to [Ubierna *et al.* \(2017\)](#), [Kolbe and Cousins \(2018\)](#), and [Sonawane and Cousins \(2019\)](#), was not significantly different between the *gdch*-KD and WT plants at 18.4 kPa pO_2 ([Supplementary Table S2](#)). The ^{18}O -based g_m is not strictly associated with the biochemistry of photosynthesis as is the ^{13}C -based g_m and therefore cannot be used to estimate f . The values of ^{13}C -based g_m for *gdch*-KD

and WT plants at each pO_2 were used to calculate the corresponding pCO_2 in the chloroplasts (C_c , Pa) by applying Fick's first law.

The Γ^* was defined in terms of Rubisco kinetic properties according to [Jordan and Ogren \(1984\)](#), and the estimate of CO_2 released per O_2 reacting with RuBP (α) was determined for the *gdch*-KD plants versus α set equal to 0.5 in the WT as described in [Supplementary Methods S5](#). A sensitivity analysis for the dependency of f on Γ^* and α is also described in [Supplementary Methods S5](#).

^{13}C composition of leaf dark-evolved CO_2 and contributions of leaf chamber and growth chamber assimilates to substrates feeding leaf dark respiration

The ^{13}C composition of the dark-evolved CO_2 determining R_d ($\delta^{13}C_{Rd}$, ‰) was calculated according to [Barbour *et al.* \(2007\)](#) as described by [Evans *et al.* \(1986\)](#).

The substrates feeding leaf dark respiration were from carbon assimilates produced in the L_{ch} and in the G_{ch} . Given $\delta^{13}C_{Rd(i)}$ as the mean values of $\delta^{13}C$ for dark-evolved CO_2 at time i from light-dark transition, the fractional contribution of L_{ch} assimilates to $\delta^{13}C_{Rd(i)}$ ($\delta^{Rd}L_{ch_substr(i)}$, ‰/‰) was calculated for *gdch*-KD and WT plant types after leaf photosynthesis under both O_2 levels as

$$\delta^{Rd}L_{ch_substr(i)} = \frac{(\delta^{13}C_{Rd(i)} - \delta^{13}C_{Rd(24h)})}{(\delta^{13}C_{Lch_Ph} - \delta^{13}C_{Rd(24h)})} \quad (1)$$

where $\delta^{13}C_{Rd(i)}$ was determined by steps of 3 min over 195 min in the dark; $\delta^{13}C_{Rd(24h)}$ is the mean $\delta^{13}C_{Rd}$ after 24 h in the dark as shown in [Supplementary Table S1](#); and $\delta^{13}C_{Lch_Ph}$ (‰) is the representative $\delta^{13}C$ of *gdch*-KD or WT carbon assimilates produced in the L_{ch} at a pO_2 of 1.84 kPa or 18.4 kPa before the light-dark transition (values are shown in [Supplementary Table S1](#)). The assumptions underlying Equation 1 and the calculation of $\delta^{13}C_{Lch_Ph}$ are reported in [Supplementary Methods S6](#).

Based on the total fractional contributions of L_{ch} and G_{ch} carbon assimilates to $\delta^{13}C_{Rd}$ equal to 1.0, the complementing fractional contribution of G_{ch} assimilates to $\delta^{13}C_{Rd(i)}$ [$\delta^{Rd}G_{ch_substr(i)}$, ‰/‰] was calculated for both plant types after leaf photosynthesis under both O_2 levels as

$$\delta^{Rd}G_{ch_substr(i)} = 1 - \delta^{Rd}L_{ch_substr(i)} \quad (2)$$

In addition, to make a combined analysis of the data collected in the two O_2 experimental conditions possible, the $\delta^{13}C_{Rd}$ generated from plants grown at the more depleted $\delta^{13}C_{Gch}$ were edited to cancel out the bias in the $\delta^{13}C_{Gch}$ effect on $\delta^{13}C_{Rd}$ with respect to the other batch of plants. In particular, the $\delta^{13}C_{Rd}$ following leaf photosynthesis at the lower O_2 experimental level were edited through the procedure described in [Supplementary Methods S7](#).

Leaf CO_2 compensation points in the presence of R_L

Leaf-atmosphere gas exchange measurements were taken with an LI-6400XT portable gas analyzer equipped with the 2×3 cm L_{ch} on *gdch*-KD and WT plants ($n=4$) at a PPFD of 1500 $\mu mol photons m^{-2} s^{-1}$, t_{leaf} of 25 °C, leaf to air VPD between 1.0 kPa and 1.5 kPa, C_a decreasing from 35.0 Pa to 3.7 Pa, and at a pO_2 of 1.84 kPa or 18.4 kPa. For each leaf, a least square regression analysis of the response of A ($\mu mol CO_2 m^{-2} s^{-1}$) to C_i (Pa) was applied to the initial slope (for $C_i \leq 9.2$ Pa) to determine the CO_2 compensation point in the presence of R_L (Γ , Pa).

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). A linear mixed effects model (PROC MIXED) was used with plant type (*gdch*-KD and WT) and O_2 level (pO_2 of 1.84 kPa and 18.4 kPa) as fixed factors and leaves as random factor nested within plant type. The effects of plant type, O_2 level, and plant type $\times O_2$ level interaction on A , g_s , C_i , C_i/C_a , Δ_o , C_c , Γ , $R_{d(6m)}$, $\delta^{13}C_{Rd(6m)}$, $R_{d(30m)}$, $\delta^{13}C_{Rd(30m)}$, $R_{d(3h)}$, and $\delta^{13}C_{Rd(3h)}$ were assessed. A PROC MIXED procedure was

Table 1. Description of the abbreviations, symbol, value (as in *Evans and von Caemmerer, 2013*), and unit of the environmental parameters and leaf variables used in this study

Abbreviation	Description	
G _{ch}	Growth chamber	
GDC	Glycine decarboxylase complex	
gdch-KD	Transgenic GDC H-subunit knockdown	
L _{ch}	Leaf chamber	
LEDR	Light-enhanced dark respiration	
NH ₃	Ammonia	
NH ₄ ⁺	Ammonium cation	
PDH	Pyruvate dehydrogenase	
RuBP	Ribulose 1,5-bisphosphate	
TCA	Tricarboxylic acid	
2-PG	2-phosphoglycolate	
3-PGA	3-phosphoglycerate	
Symbol	Environmental parameters/leaf variables	Value and unit
A	Net CO ₂ assimilation rate per unit (one side) leaf surface area	μmol CO ₂ m ⁻² s ⁻¹
a	¹³ C fractionation during CO ₂ diffusion (in air) through stomata	4.4‰
b ₃	Rubisco ¹³ C fractionation	29.0‰
C _a	CO ₂ mole fraction or CO ₂ partial pressure set in the leaf chamber	μmol mol ⁻¹ ; Pa
C _c	CO ₂ mole fraction or CO ₂ partial pressure in the chloroplast	μmol mol ⁻¹ ; Pa
C _i	CO ₂ mole fraction or CO ₂ partial pressure in the intercellular air space	μmol mol ⁻¹ ; Pa
C _{in}	CO ₂ mole fraction entering the leaf chamber	μmol mol ⁻¹
C _{out}	CO ₂ mole fraction leaving the leaf chamber	μmol mol ⁻¹
C _s	CO ₂ mole fraction at the leaf surface	μmol mol ⁻¹
f	Photorespiratory ¹³ CO ₂ fractionation	‰
g _m	Mesophyll conductance to CO ₂ diffusion from the substomatal cavity to the chloroplast stroma	μmol CO ₂ m ⁻² s ⁻¹ Pa ⁻¹
g _{sc}	Stomatal conductance to CO ₂ diffusion	μmol CO ₂ m ⁻² s ⁻¹ Pa ⁻¹
LMA	Leaf dry mass per (one side) unit surface area	g m ⁻²
pCO ₂	Partial pressure of CO ₂	Pa
pO ₂	Partial pressure of O ₂	kPa
PPFD	Photosynthetic photon flux density	μmol photons m ⁻² s ⁻¹
R _d	Dark respiration rate per unit (one side) leaf surface area	μmol CO ₂ m ⁻² s ⁻¹
R _{d(24h)}	R _d after 24 h dark	μmol CO ₂ m ⁻² s ⁻¹
R _{d(30min)}	R _d after 30 min dark	μmol CO ₂ m ⁻² s ⁻¹
R _{d(3h)}	R _d after 3 h dark	μmol CO ₂ m ⁻² s ⁻¹
R _{d(6min)}	R _d after 6 min dark	μmol CO ₂ m ⁻² s ⁻¹
R _l	Light mitochondrial non-photorespiratory respiration rate per unit (one side) leaf surface area	μmol CO ₂ m ⁻² s ⁻¹
t	Correction factor for ternary effects	‰
t _{air}	Air temperature	°C
t _{leaf}	Leaf temperature	°C
VPD	Vapor pressure deficit	kPa
α	Moles of CO ₂ released in the photorespiratory pathway per mole of O ₂ reacting with RuBP	mol CO ₂ mol ⁻¹ O ₂
Δ _e	¹³ C discrimination associated with mitochondrial non-photorespiratory respiration	‰
Δ _f	¹³ C discrimination associated with photorespiration	‰
Δ _{gm}	¹³ C discrimination associated with mesophyll conductance to CO ₂ diffusion	‰
Δ _i	¹³ C discrimination due to carboxylation, boundary layer and stomatal CO ₂ diffusion	‰
Δ _o	Observed (instantaneous) leaf net discrimination against ¹³ CO ₂ in the light	‰
Γ	CO ₂ compensation point	μmol mol ⁻¹ ; Pa
Γ*	CO ₂ compensation point in absence of mitochondrial non-photorespiratory respiration	μmol mol ⁻¹ ; Pa
δ _{in}	δ ¹³ C of CO ₂ entering the leaf chamber	‰
δ _{out}	δ ¹³ C of CO ₂ leaving the leaf chamber	‰
δ ¹³ Rd _{G_{ch}_substr}	Fractional contribution of respiratory substrates from G _{ch} carbon assimilates to δ ¹³ C of dark-evolved CO ₂	‰/‰
δ ¹³ Rd _{L_{ch}_substr}	Fractional contribution of respiratory substrates from L _{ch} carbon assimilates to δ ¹³ C of dark-evolved CO ₂	‰/‰
δ ¹³ C	¹³ C composition of CO ₂	‰
δ ¹³ C _{dm}	¹³ C signature of leaf dry matter	‰
δ ¹³ C _{Gch}	¹³ C composition of atmospheric CO ₂ in the growth chamber during the photoperiod	‰
δ ¹³ C _{Lch_Ph}	Representative δ ¹³ C of carbon assimilates produced in the L _{ch}	‰
δ ¹³ C _{Rd}	δ ¹³ C of CO ₂ evolved by leaves in the dark	‰
δ ¹³ C _{Rd(24h)}	δ ¹³ C of CO ₂ evolved by leaves after 24 h dark	‰
δ ¹³ C _{Rd(30min)}	δ ¹³ C of CO ₂ evolved by leaves after 30 min dark	‰
δ ¹³ C _{Rd(3h)}	δ ¹³ C of CO ₂ evolved by leaves after 3 h dark	‰
δ ¹³ C _{Rd(6min)}	δ ¹³ C of CO ₂ evolved by leaves after 6 min dark	‰

applied as a one-way ANOVA to determine the plant type (fixed factor) effect on the following traits: total N content, malate content, Γ^* , Δ_o , Δ_i , $\Delta_i - \Delta_o$, Δ_{gm} , Δ_e , Δ_p , $R_d(24h)$, $\delta^{13}C_{Rd(24h)}$, LMA, and $\delta^{13}C_{dm}$. A one-sample *t*-test ($P < 0.05$) was applied to test the difference of *f* or α modeled for the *gdch*-KD plants compared with a constant *f* or α value assumed in the WT. A two-sample *t*-test ($P < 0.05$) was applied to test the difference between *gdch*-KD and WT g_m at a pO_2 of 1.84 kPa, and WT g_m at the two O_2 levels. For each plant type, a three-parameter non-linear model was fit to the R_d and $\delta^{13}C_{Rd}$ responses determined over the 195 min in the dark after leaf photosynthesis at each O_2 experimental level. In particular, the $\delta^{13}C_{Rd}$ values associated with the lower O_2 level had been first edited as described in Supplementary Methods S7, and then used for the analysis. The fitting model $y = \theta_1 e^{-\theta_2 x} + \theta_3$ was employed where x are minutes from 0 to 195 by steps of three, and y are R_d or $\delta^{13}C_{Rd}$ values; θ_1 , θ_2 , and θ_3 are the range, slope, and lower asymptote (or floor) parameters, respectively, which were determined using non-linear least squares with the iterative Gauss–Newton algorithm. Specifically, for R_d or $\delta^{13}C_{Rd}$ responses, the range parameter corresponds to the difference between initial and lower asymptote values, and the slope is the exponential rate of change. An extra sum of squares *F*-test was applied to define the significance ($P < 0.05$) of the effects of plant type and O_2 level (main effects), and plant type \times O_2 level interactions on the three parameters of R_d or $\delta^{13}C_{Rd}$ fitting models.

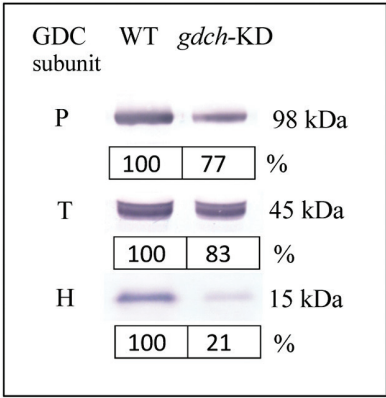


Fig. 1. Immunoblot analysis for GDC P-, T-, and H-subunits in mature leaves of *gdch*-KD compared with WT plants. The protein molecular weight of each subunit (kDa) is shown. Subunit protein abundances for *gdch*-KD plants are mean percentage values of the WT ($n=2$).

Results

Leaf GDC protein and malate content

Leaves of *gdch*-KD plants had 21% (± 2 SE) H-protein content compared with the WT, while the P- and T-protein content was 77% (± 6 SE) and 83% (± 2 SE) of that of the WT, respectively (Fig. 1; $n=2$). Malate content in leaf samples taken immediately after measurements of leaf photosynthesis were 0.49 ± 0.08 mmol m^{-2} and 0.38 ± 0.02 mmol m^{-2} (mean \pm SE; $n=5$) in *gdch*-KD and WT plants, respectively ($P=0.29$).

Leaf physiological analysis

Leaf photosynthetic responses

There was no observable difference in growth phenotypes between the *gdch*-KD and WT plants when they were grown under 184.2 Pa pCO_2 (2000 μ mol mol^{-1}). However, at approximately current ambient CO_2 and O_2 partial pressures, the net rate of CO_2 assimilation (*A*) was lower in the *gdch*-KD compared with the WT but there was no significant difference in *A* between plant types under low photorespiratory conditions, when pO_2 was reduced to 1.84 kPa (Table 2). There was, however, a significant effect of O_2 level on g_{sc} (negative effect) and C_i/C_a (positive effect) but not a plant type effect (Table 2). There was a significant plant type \times O_2 level interaction on Δ_o , which showed higher values for the *gdch*-KD compared with the WT at a pO_2 of 18.4 kPa, but no difference at a pO_2 of 1.84 kPa (Table 2). There was no significant plant type effect on g_m at a pO_2 of 1.84 kPa ($P=0.586$), and no O_2 effect on g_m in the WT ($P=0.701$; Table 2). There was, however, a significant effect of plant type on C_c , which showed comparable values in the *gdch*-KD and WT plants at 1.84 kPa pO_2 and higher values in the *gdch*-KD plants compared with the WT at 18.4 kPa pO_2 (modeled based on equal g_m in both transgenic and WT plants). In addition, O_2 level had a positive effect on C_c (Table 2). The Γ in the *gdch*-KD compared with WT plants was significantly lower under 1.84 kPa pO_2 but higher under 18.4 kPa pO_2 (Table 3). There was no significant difference in

Table 2. Leaf photosynthetic traits estimated on *gdch*-KD and WT plants under approximately current ambient and below current ambient O_2 levels (pO_2 of 18.4 kPa and 1.84 kPa, respectively) at C_a of 27.6 Pa.

	pO_2	<i>A</i>	g_{sc}	C_i		g_m^a	C_c	Δ_o
	(kPa)	(μ mol CO_2 m^{-2} s^{-1})	(μ mol CO_2 m^{-2} s^{-1} Pa^{-1})	(Pa)	C_i/C_a	(μ mol CO_2 m^{-2} s^{-1} Pa^{-1})	(Pa)	(‰)
<i>gdch</i> -KD	1.84	24.7 \pm 1.4	3.47 \pm 0.53	18.1 \pm 0.8	0.66 \pm 0.03	4.56 \pm 0.43	12.6 \pm 1.4	14.9 \pm 0.7
	18.4	6.3 \pm 0.3	1.37 \pm 0.11	22.1 \pm 0.2	0.80 \pm 0.01		20.6 \pm 0.2	23.7 \pm 0.5
WT	1.84	21.6 \pm 1.2	2.78 \pm 0.43	18.5 \pm 0.9	0.67 \pm 0.03	3.80 \pm 0.76	12.6 \pm 0.5	14.3 \pm 0.8
	18.4	14.3 \pm 0.8	2.45 \pm 0.31	20.5 \pm 0.5	0.74 \pm 0.02	4.07 \pm 0.14	17.0 \pm 0.6	17.8 \pm 0.3
Significance	Plant type	$P=0.050$	$P=0.629$	$P=0.411$	$P=0.411$	–	$P=0.031$	$P=0.003$
	pO_2	$P<0.001$	$P=0.018$	$P=0.003$	$P=0.003$	–	$P=0.000$	$P<0.001$
	Plant	$P=0.002$	$P=0.057$	$P=0.171$	$P=0.171$	–	$P=0.033$	$P=0.003$
	type \times pO_2							

Values are the mean \pm SE ($n=4$). Significance ($P < 0.05$) of the effects of plant type, pO_2 , and plant type \times pO_2 interaction were evaluated by SAS PROC MIXED.
^a No significant differences were evaluated by a two sample *t*-test (significance for $P < 0.05$) between the *gdch*-KD and WT g_m values at a pO_2 of 1.84 kPa ($P=0.586$), and the WT g_m values at the two pO_2 values ($P=0.701$).

leaf N content between plant types, with means of 2.30 ± 0.08 SE g m⁻² and 2.31 ± 0.14 SE g m⁻² in *gdch*-KD and WT leaves, respectively ($n=4$).

Δ_o plotted versus C_i/C_a showed a similar response in the *gdch*-KD and WT plants at 1.84 kPa pO_2 but was significantly greater in the *gdch*-KD compared with WT plants at 18.4 kPa pO_2 (Fig. 2; see Table 2 for statistical analysis). The *gdch*-KD plants had significantly lower Δ_{gm} , Δ_f and Δ_e compared with the WT under a pO_2 of 18.4 kPa (Fig. 3A). Additionally, the *gdch*-KD plants had a significantly lower f with mean values of $3.4 \pm 0.5\%$ SE ($n=4$) compared with 16.2% in the WT under approximately current ambient pO_2 (Fig. 3B; $P<0.001$). A significantly higher α was determined at 18.4 kPa pO_2 in *gdch*-KD plants, with a mean value of 0.59 ± 0.01 SE ($n=3$), versus 0.5 assumed for the WT ($P<0.01$). There was a negative linear dependency of f on Γ^* and on α (Supplementary Methods S5; Fig. S1A and B, respectively); however, there was a positive linear dependency of f on g_m and R_L , with a greater sensitivity to g_m (Supplementary Fig. S2A and B, respectively).

Leaf dark respiration responses

In the *gdch*-KD and WT plants, the R_d showed a hyperbolic decrease over the 3 h in the dark after leaf light exposure under different O₂ levels, with a noticeable rapid decline in the first hour; however, R_d was higher following leaf photosynthesis at a pO_2 of 18.4 kPa compared with 1.84 kPa (Fig. 4). A significant positive O₂ effect on R_d responses of *gdch*-KD and WT plants was inferred based on significantly higher floor ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; $P<0.0001$) and range ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; $P<0.0001$) parameters after leaf photosynthesis at pO_2 of 18.4 kPa compared with 1.84 kPa in the non-linear model fit to the R_d responses (Supplementary Tables S3, S4). In particular, based on the spot measurements, a significant positive O₂ effect was determined on $R_{d(6\text{min})}$ (together with a significant plant type effect), $R_{d(30\text{min})}$, and $R_{d(3h)}$ (Table 4). A significant plant type effect on R_d responses was inferred based on a statistically larger range ($P=0.023$) and less steep rate of exponential change (slope, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ min}^{-1}$; $P<0.0001$) for the *gdch*-KD versus WT plants (Supplementary Tables S3, S4). After leaf photosynthesis at a pO_2 of 18.4 kPa, a significant plant

type effect on R_d (with higher R_d determined in the *gdch*-KD plants versus the WT; see Fig. 4) was driven by the significantly less steep R_d slope ($P<0.001$) in *gdch*-KD compared with WT plants. In addition, following leaf photosynthesis at a pO_2 of 18.4 kPa, a change in R_d for $\sim 75\%$ of the R_d range occurred in WT plants within the first 30 min after light–dark transition; in contrast, this fractional variation took ~ 90 min in the *gdch*-KD plants (Fig. 4). The mean values of R_L inferred from $R_{d(3h)}$ were 0.59 ± 0.03 SE $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *gdch*-KD and 0.56 ± 0.03 SE $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for WT plants after leaf photosynthesis under a pO_2 of 1.84 kPa ($n=4$). In contrast, R_L was 0.98 ± 0.12 SE $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *gdch*-KD and 0.82 ± 0.09 SE $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for WT plants after leaf exposure to a pO_2 of 18.4 kPa ($n=4$). For the R_L values, a non-significant plant type effect and a significant effect of the O₂ level can be inferred from the significance of $R_{d(3h)}$ (see Table 4). In addition, no significant difference in leaf dry mass per unit surface area (LMA) was determined between *gdch*-KD and WT plants, with values of 43.6 ± 2.7 SE g m⁻² and 44.5 ± 1.5 SE g m⁻² ($n=4$), respectively.

In *gdch*-KD and WT plants, the $\delta^{13}\text{C}_{Rd}$ estimated over the 3 h after light–dark transition showed a negative hyperbolic pattern, with most of the $\delta^{13}\text{C}_{Rd}$ variation occurring in the first 30 min (Fig. 5A, B). A tight positive correlation between R_d and $\delta^{13}\text{C}_{Rd}$ over the 3 h dark period was determined after leaf photosynthesis at a pO_2 of 1.84 kPa for both plant types ($r>0.90$). After leaf photosynthesis at a pO_2 of 18.4 kPa, a positive correlation between R_d and $\delta^{13}\text{C}_{Rd}$ with $r=0.75$ and $r=0.78$ was determined for *gdch*-KD and the WT, respectively. Statistical analysis of a non-linear model fit to the $\delta^{13}\text{C}_{Rd}$ responses showed a significantly lower $\delta^{13}\text{C}_{Rd}$ range after leaf photosynthesis at a pO_2 of 18.4 kPa ($\%$; $P<0.0001$) compared with 1.84 kPa pO_2 . In contrast, the floor parameter was non-significantly different between the O₂ levels (Supplementary Tables S5, S6). These statistical results indicate a significant effect of the O₂ level during previous leaf light exposure on

Table 3. CO₂ compensation points (Γ) determined under low photorespiratory (1.84 kPa pO_2) and photorespiratory (18.4 kPa pO_2) conditions on *gdch*-KD and WT plants

Plant-type	pO_2 (kPa)	Γ (Pa)
<i>gdch</i> -KD	1.84	0.25 ± 0.06
	18.4	5.48 ± 0.04
WT	1.84	0.62 ± 0.12
	18.4	4.54 ± 0.18
Significance	Plant type	$P=0.055$
	pO_2	$P<0.001$
	Plant type $\times pO_2$	$P=0.001$

Values are the mean \pm SE ($n=3$ for *gdch*-KD at a pO_2 of 18.4 kPa; $n=4$ otherwise). Significance ($P<0.05$) for the effects of plant type, pO_2 , and plant type $\times pO_2$ interaction was evaluated by SAS PROC MIXED.

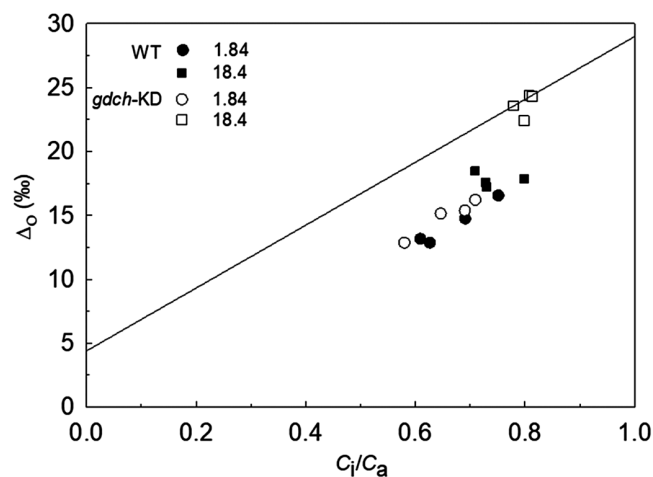


Fig. 2. Leaf ¹³CO₂ net discrimination in the light (Δ_o) versus C_i/C_a under a pO_2 of 1.84 kPa and 18.4 kPa for individual *gdch*-KD and WT plants. The line represents the leaf ¹³CO₂ net discrimination modeled in relation to C_i/C_a as $\Delta^{13}\text{C}_{mod} = a + (b_3 - a) \times C_i/C_a$ (Farquhar *et al.*, 1982) where $a=4.4\%$ and $b_3=29.0\%$. $\Delta^{13}\text{C}_{mod}$ is a proxy of Δ_i as described by Evans and von Caemmerer (2013). Open symbols are for *gdch*-KD and filled symbols for WT plants. Circles are for a pO_2 of 1.84 kPa and squares for a pO_2 of 18.4 kPa.

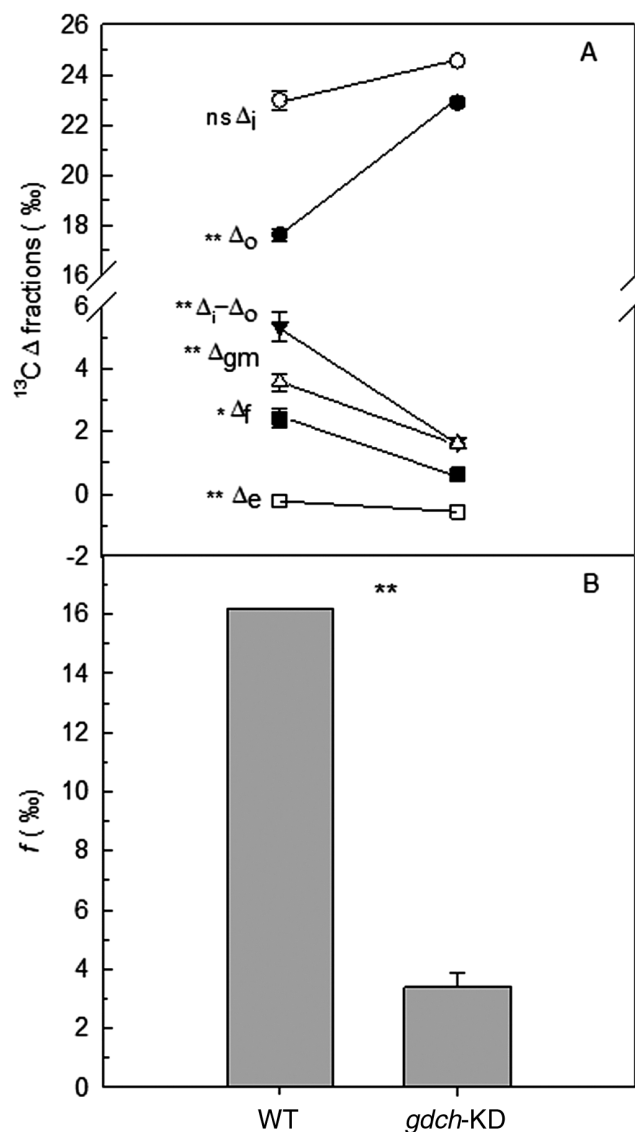


Fig. 3. Leaf $^{13}\text{CO}_2$ net discrimination and discrimination fractions in the light, and $^{13}\text{CO}_2$ photorespiratory fractionation for $gdch\text{-KD}$ versus WT plants determined based on Evans and von Caemmerer (2013). (A) Observed leaf net $^{13}\text{CO}_2$ discrimination in the light (Δ_o), and modeled ^{13}C discrimination fractions for $gdch\text{-KD}$ ($n=3$) and the WT ($n=4$) at an atmospheric $p\text{O}_2$ of 18.4 kPa. Δ_i is the additive $^{13}\text{CO}_2$ discrimination during CO_2 diffusion from atmosphere to intercellular air space and due to carboxylation; $\Delta_i - \Delta_o$ is comprised of three terms: Δ_{gm} , which is the $^{13}\text{CO}_2$ fractionation fraction during CO_2 diffusion in the liquid phase to chloroplast stroma, and Δ_e and Δ_f , which are the ^{13}C fractionation fractions associated with light mitochondrial non-photorespiratory respiration and photorespiration, respectively. Δ_f was calculated as $\Delta_f = \Delta_i - \Delta_o - \Delta_{gm} - \Delta_e$. Values are mean \pm SE. (B) $^{13}\text{CO}_2$ fractionation for photorespiration (f) in $gdch\text{-KD}$ plants calculated at an atmospheric $p\text{O}_2$ of 18.4 kPa from $\Delta_f = \frac{1+t}{1-t} \left(f_{\text{C}_3}^{13} \right)$ versus f of 16.2‰ in the WT. Values for $gdch\text{-KD}$ plants are the mean \pm SE ($n=3$). Significance ($P < 0.05$) of the effect of plant type on the variables in (A) was evaluated by SAS PROC MIXED as a one-way ANOVA; * for $0.01 < P < 0.05$; ** for $P < 0.01$. Significance in (B) was evaluated by one-sample t-test ($P < 0.05$). ** for $P < 0.01$.

the $\delta^{13}\text{C}_{\text{Rd}}$ values of both plant types over 3 h in the dark (with lower $\delta^{13}\text{C}_{\text{Rd}}$ at a $p\text{O}_2$ of 18.4 kPa, and higher $\delta^{13}\text{C}_{\text{Rd}}$ at a $p\text{O}_2$ of 1.84 kPa). The spot $\delta^{13}\text{C}_{\text{Rd}}$ measurements also showed significantly lower $\delta^{13}\text{C}_{\text{Rd}(6\text{min})}$ and $\delta^{13}\text{C}_{\text{Rd}(30\text{min})}$ after leaf photosynthesis at a $p\text{O}_2$ of 18.4 kPa compared with 1.84 kPa (Table

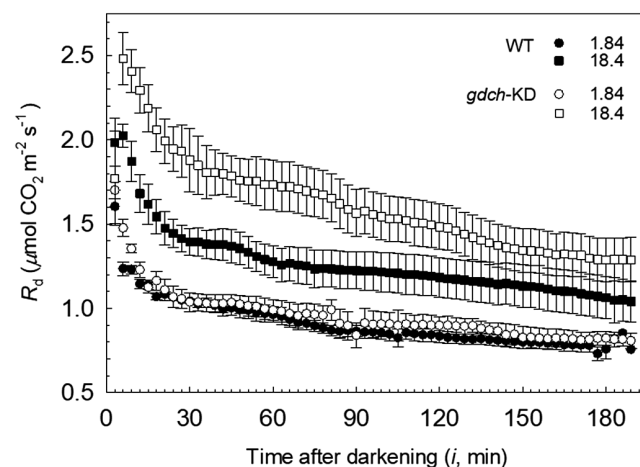


Fig. 4. Dynamics of leaf dark respiration rate (R_d) determined during ~3 h in the dark on $gdch\text{-KD}$ (open symbols) and WT (filled symbols) plants after leaf photosynthesis under a $p\text{O}_2$ of 1.84 kPa (circles) or 18.4 kPa (squares). Symbols correspond to the mean \pm SE ($n=4$) determined every 3 min.

4). There was no significant plant type effect on the $\delta^{13}\text{C}_{\text{Rd}}$ over the 3 h in the dark (Table 4; Supplementary Table S6) and there was no difference for $\delta^{13}\text{C}_{\text{dm}}$ between $gdch\text{-KD}$ and the WT (Supplementary Table S1).

Over the 3 h after the light–dark transition, the fractional contribution of L_{ch} assimilates to $\delta^{13}\text{C}_{\text{Rd}}$ ($\delta^{\text{Rd}}L_{\text{ch_substr}}$, ‰/‰) showed a decreasing hyperbolic pattern for both $gdch\text{-KD}$ and WT plants (Fig. 6), with no significant differences between plant types and O_2 levels.

Discussion

Altered photorespiratory metabolism and leaf photosynthetic traits

Based on leaf protein analysis, $gdch\text{-KD}$ plants had ~21, 77, and 83% of GDC H-, P-, and T-protein abundance, respectively, compared with the WT. Previous studies reported how GDC activity is linearly correlated with H-protein accumulation (Wingler et al., 1997; Lin et al., 2016). Additionally, in agreement with Lin et al. (2016), the $gdch\text{-KD}$ plants in the current study showed an expected photorespiratory-deficient phenotype. Under photorespiratory conditions, a disruption of the photorespiratory pathway negatively affects the rate of net CO_2 assimilation (A) due to accumulation of metabolites that inhibit the Calvin–Benson cycle and restrict RuBP regeneration (Wingler et al., 2000). Specifically, leaf glycine level is a sensitive indicator of altered photorespiratory carbon flow (Blackwell et al., 1988; Timm et al., 2012). For example, $gdch\text{-KD}$ mutants of Arabidopsis, barley, and rice had substantial increases in leaf contents of glycine under ambient $p\text{O}_2$ (Bauwe and Kolukisaoglu, 2003; Lin et al., 2016). This accumulation of glycine and its precursors (P-glycolate, glycolate, and glyoxylate) in the $gdch\text{-KD}$ plants has been suggested to alter photorespiratory carbon metabolism (Peterhansel et al., 2010, 2013a). These changes have important implications for understanding and modeling leaf carbon metabolism, because they

Table 4. Leaf dark respiration rates (R_d) at 30 °C and $^{13}\text{CO}_2$ composition of dark-evolved CO_2 ($\delta^{13}\text{C}_{\text{Rd}}$) determined on *gdch*-KD versus the WT after 6 min [$R_{d(6\text{min})}$ and $\delta^{13}\text{C}_{\text{Rd}(6\text{min})}$; $n=4$], 30 min [$R_{d(30\text{min})}$ and $\delta^{13}\text{C}_{\text{Rd}(30\text{min})}$; $n=4$], 3 h [$R_{d(3\text{h})}$ and $\delta^{13}\text{C}_{\text{Rd}(3\text{h})}$; $n=4$], and 24 h [$R_{d(24\text{h})}$ and $\delta^{13}\text{C}_{\text{Rd}(24\text{h})}$; $n=3$] in the dark following leaf exposure to light under approximately current ambient and below current ambient O_2 levels ($p\text{O}_2$ of 18.4 kPa and 1.84 kPa, respectively)

Plant-type	$p\text{O}_2$ (kPa)	$R_{d(6\text{min})}$ ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	$\delta^{13}\text{C}_{\text{Rd}(6\text{min})}$ (‰)	$R_{d(30\text{min})}$ ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	$\delta^{13}\text{C}_{\text{Rd}(30\text{min})}$ (‰)	$R_{d(3\text{h})}$ ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	$\delta^{13}\text{C}_{\text{Rd}(3\text{h})}$ (‰)	$R_{d(24\text{h})}$ ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	$\delta^{13}\text{C}_{\text{Rd}(24\text{h})}$ (‰)
<i>gdch</i> -KD	1.84	1.48±0.05	-39.2±0.9*	1.04±0.06	-47.3±1.6*	0.81±0.04	-56.0±0.7*	0.79±0.02	-58.0±0.5*
WT	1.84	1.23±0.04	-40.6±1.2*	1.04±0.01	-47.2±1.7*	0.77±0.04	-54.1±0.7*	0.69±0.02	-58.6±1.0*
Significance								$P=0.045$	$P=0.705$
<i>gdch</i> -KD	18.4	2.59±0.29	-45.6±1.7	1.98±0.20	-54.1±0.9	1.34±0.17	-55.6±1.2	0.69±0.06	-58.1±0.1
WT	18.4	2.13±0.07	-43.2±0.9	1.47±0.08	-50.4±2.8	1.12±0.13	-52.5±1.8	0.74±0.08	-58.6±0.6
Significance	Plant type	$P=0.042$	$P=0.723$	$P=0.110$	$P=0.349$	$P=0.215$	$P=0.132$	$P=0.596$	$P=0.410$
	$p\text{O}_2$	$P=0.0001$	$P=0.032$	$P=0.0009$	$P=0.035$	$P=0.004$	$P=0.429$	–	–
	Plant	$P=0.407$	$P=0.238$	$P=0.069$	$P=0.375$	$P=0.326$	$P=0.753$	–	–
	type× $p\text{O}_2$								

Values are the mean ±SE; the asterisks indicate means from $\delta^{13}\text{C}_{\text{Rd}}$ values edited according to [Supplementary Method S7](#). Significance ($P<0.05$) of the effects of plant type, $p\text{O}_2$, and plant type× $p\text{O}_2$ interaction was evaluated by SAS PROC MIXED. The effect of plant type on $R_{d(24\text{h})}$ and $\delta^{13}\text{C}_{\text{Rd}(24\text{h})}$ was evaluated at a $p\text{O}_2$ of 1.84 kPa or 18.4 kPa by one-way ANOVA (significance for $P<0.05$).

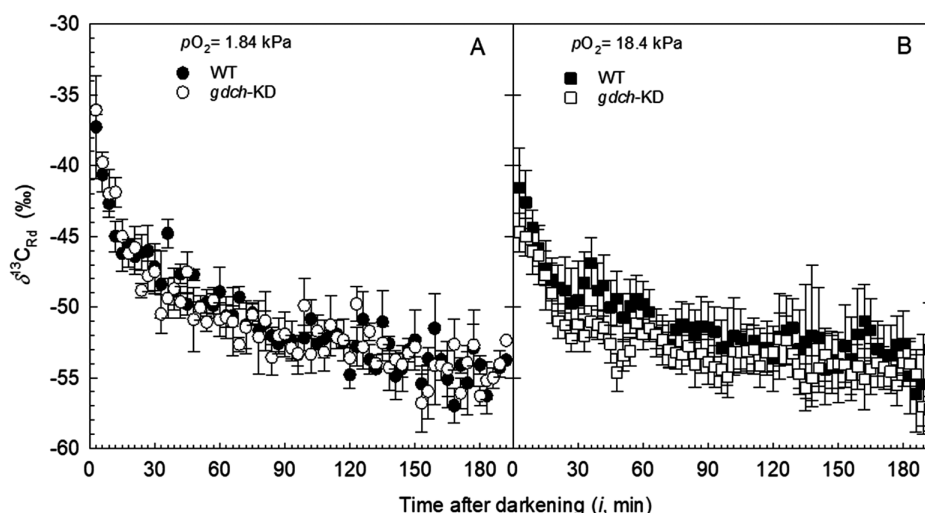


Fig. 5. $^{13}\text{CO}_2$ composition associated with R_d ($\delta^{13}\text{C}_{\text{Rd}}$) determined during ~3 h in the dark in *gdch*-KD (open symbols) and WT (filled symbols) plants. (A) $\delta^{13}\text{C}_{\text{Rd}}$ after leaf photosynthesis under a $p\text{O}_2$ of 1.84 kPa edited (see [Supplementary Methods S7](#)) to remove the effect of a lower atmospheric $\delta^{13}\text{CO}_2$ compared with (B) while growing the plants. (B) Distributions of $\delta^{13}\text{C}_{\text{Rd}}$ after leaf photosynthesis under a $p\text{O}_2$ of 18.4 kPa. Symbols correspond to the mean ±SE ($n=4$) determined every 3 min.

may influence the stoichiometry of CO_2 released per oxygenation reaction (α) and the CO_2 compensation point (Γ) (see [Cousins et al., 2008, 2011; Walker and Cousins, 2013](#)).

In the present study, the *gdch*-KD plants had greater Γ compared with the WT under 18.4 kPa $p\text{O}_2$, as previously reported by [Lin et al. \(2016\)](#). This may be partially due to enhanced R_L , which [Igamberdiev et al. \(2004\)](#) and [Bykova et al. \(2005\)](#) reported was needed to compensate for the lack of photorespiratory regulation of redox and energy balance ([Igamberdiev et al., 2001](#)). The increase in Γ in the *gdch*-KD plants could also be associated with a higher α leading to an increasing Γ^* compared with the WT. It has been previously suggested

that α increased in Arabidopsis mutants lacking peroxysomal hydroxypyruvate reductase ([Cousins et al., 2008](#)) and the peroxysomal malate dehydrogenase ([Cousins et al., 2011](#)). However, these previous publications did not determine whether these disruptions to photorespiration influenced leaf CO_2 isotope exchange.

Leaf net ^{13}C discrimination in the light and photorespiratory ^{13}C fractionation

Under leaf photorespiratory conditions, the change in leaf net discrimination against $^{13}\text{CO}_2$ (Δ_o) in the *gdch*-KD plants

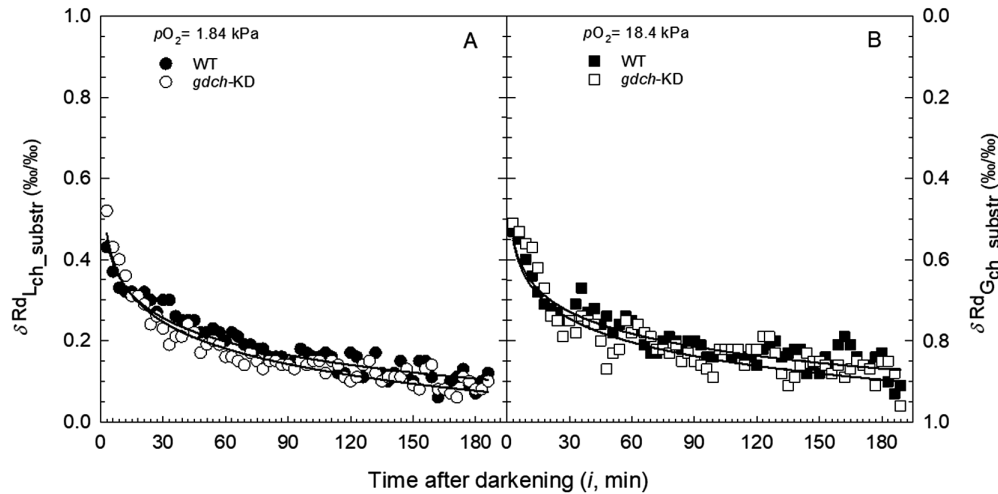


Fig. 6. Distributions over ~3 h in the dark of the fractional contributions (total contribution equal to 1.0) to $\delta^{13}\text{C}_{\text{Rd}}$ of recent L_{ch} carbon assimilates ($\delta^{13}\text{C}_{\text{Rd_Lch_substr}}$, ‰/‰) and G_{ch} assimilates ($\delta^{13}\text{C}_{\text{Rd_Gch_substr}}$, ‰/‰) for *gdch*-KD (open symbols) and WT (filled symbols) after leaf light exposure at a $p\text{O}_2$ of (A) 1.84 kPa and (B) 18.4 kPa. The first values are at 3 min after light–dark transition. Symbols correspond to the mean determined every 3 min ($n=4$). Continuous lines represent logarithmic trend lines ($R^2 > 0.90$) for *gdch*-KD (lower) and the WT (higher), respectively.

compared with WT plants was caused by a higher C_i/C_a , lower Δ_{gm} , greater Δ_e , and lower Δ_f (Fig. 3A). However, the lower Δ_{gm} in the *gdch*-KD plants with respect to the WT was due to a reduction in A , since WT g_m was applied to both plant types (see Δ_{gm} equation in Evans and von Caemmerer, 2013). In addition, a difference in Δ_e between *gdch*-KD and WT plants was related to proportional changes in $R_L/(A+R_L)$ and $(C_i-\Gamma^*)$ (see Δ_e equation in Evans and von Caemmerer, 2013).

The term Δ_f is dependent on $\frac{\Gamma^*}{C_i}$, but, despite the higher Γ^* in the *gdch*-KD relative to WT plants, it was lower in the transgenic plants caused by the lower f . In WT plants, f is primarily attributed to the ^{13}C discrimination associated with the decarboxylation of glycine catalyzed by GDC (Tcherkez *et al.*, 2004; Tcherkez, 2006). While Rooney (1988) determined an *in vitro* f of 7–8‰ for *Glycine max* (soybean), Igamberdiev *et al.* (2001, 2004) reported f for several species between 9.8 and 13.7‰, and Ghashghaie *et al.* (2003) reported an f of >9–11‰ for *Senecio* species. Additionally, Evans and von Caemmerer (2013) determined an *in vivo* f of 16.2‰ in *Nicotiana tabacum*. Based on Farquhar *et al.* (1982), and according to O’Leary (1988) and Tcherkez (2006),

$$f = \frac{(\delta^{13}\text{C}_{\text{glycine}} - \delta^{13}\text{C}_{\text{photorespired_CO}_2})}{(1 + \delta^{13}\text{C}_{\text{photorespired_CO}_2}/1000)} \quad (3)$$

where glycine is assumed to have the same ^{13}C signature of recently fixed carbon. Therefore, an increase in $\delta^{13}\text{C}$ of the released CO_2 during photorespiration corresponds to a linear decrease in f . There is also a negative linear dependency of f on Γ^* and α , as shown in Supplementary Methods S5; Fig. S1A, B.

In C_3 plants, most of the CO_2 released by photorespiration tends to be through GDC (Badger, 1985; Bauwe *et al.*, 2010). However, previous reports have suggested that alternative reactions can release CO_2 when the flux of glycolate into the photorespiratory cycle exceeds its metabolic capacity, or when the traditional photorespiratory pathway has been genetically disrupted (Cousins *et al.*, 2008, 2011; Timm *et al.*,

2008; Peterhansel *et al.*, 2013a). The GDC multienzyme system requires all subunits to function (Douce *et al.*, 2001); in the present study, since a low level of the H-subunit was determined in *gdch*-KD plants, some residual activity for GDC is expected in the transgenic plants. A change in ^{13}C fractionation associated with the knockdown of GDC activity is therefore unlikely because the products of the glycine decarboxylation reaction (NH_4^+ , NADH, and methylene-tetrahydrofolate) can be readily processed by downstream reactions in the glycolate pathway (Bauwe *et al.*, 2010; Maurino and Peterhansel, 2010). Thus, the higher α and lower f in the *gdch*-KD compared with the WT suggest an increased flow of photorespiratory carbon through alternative decarboxylation reactions, independent of the GDC, and a buildup of photorespiratory metabolites.

Leaf dark respiration and ^{13}C isotopic composition of dark-evolved CO_2

Leaves of C_3 plants in the first 30 min after light–dark transition largely respire metabolites (carbohydrates and organic acids) recently produced in the light (Cornic, 1973; Rademacher *et al.*, 2002; Barbour *et al.*, 2007; Werner *et al.*, 2009; Werner and Gessler, 2011; Lehmann *et al.*, 2015, 2016) and show high rates of CO_2 evolution (named as LEDR, light-enhanced dark respiration; Atkin *et al.*, 1998). While the activity of pyruvate dehydrogenase (PDH) and metabolism in the tricarboxylic acid (TCA) cycle are the major mitochondrial decarboxylations in the dark, they are partially inhibited in the light (Ghashghaie *et al.*, 2003; Tcherkez *et al.*, 2005, 2008; Barbour *et al.*, 2007). It has been suggested that LEDR mostly depends on a buildup of malate and fumarate in the light, which are then rapidly decarboxylated after the light–dark transition (Atkin *et al.*, 1998; Barbour *et al.*, 2007; Tcherkez *et al.*, 2012). However, there is evidence for species-specific differences (Lehmann *et al.* 2016; Gessler *et al.*, 2017). Overall, R_d in the *gdch*-KD and WT plants showed an expected negative hyperbolic pattern during 3 h in the dark following leaf photosynthesis. The results of the

present study indicate that leaf photosynthesis under photorespiring conditions, before the light–dark transition, led to an additional buildup of TCA cycle substrates in the *gdch*-KD plants compared with the WT. In fact, the *gdch*-KD plants had a significantly higher R_d over 3 h dark following leaf photosynthesis under the approximately current ambient O₂ level (with leaf blades having no significantly different LMA), compared with the WT; this suggests a greater accumulation of metabolites in the light, in particular photorespiratory intermediates, as respiratory substrates to feed R_d . More precisely, a restricted photorespiratory pathway in the light may lead to an accumulation of 2-carbon metabolites in the *gdch*-KD plants.

The increase in LEDR has been reported to come from the decarboxylation of ¹³C heavier metabolites, primarily malate, and the decline in LEDR rates and $\delta^{13}\text{C}_{Rd}$ over time due to a decrease in malate availability (Barbour *et al.* 2007; Gessler *et al.*, 2009). In the *gdch*-KD plants, the cumulative leaf respired CO₂ over 30 min after leaf photosynthesis at a $p\text{O}_2$ of 18.4 kPa was 4.1 mmol CO₂ m⁻² higher with respect to the WT; theoretically, if this enhancement of R_d in the *gdch*-KD plants was due to malate alone this would require ~1 mmol malate m⁻². The non-significant differences in the leaf malate content determined during the light period between *gdch*-KD and the WT suggest that metabolites other than malate, such as photorespiratory intermediates, may have contributed to the greater LEDR rates in the *gdch*-KD plants compared with the WT. A substantial part of the malate in leaves is also stored in vacuoles, as observed in C₄ plants (Hatch, 1979; Arrivault *et al.* 2017), and not readily available for LEDR.

In the *gdch*-KD and WT plants presented here, the $\delta^{13}\text{C}_{Rd}$ decreased during the 3 h dark period, tracking the decline in R_d (Fig. 5A, B). Over the 3 h of darkness, there was an increase in the contribution to $\delta^{13}\text{C}_{Rd}$ from respiratory substrates generated during plant growth ($\delta^{13}\text{C}_{\text{ch_substr}}$, ‰/‰) for both plant types and O₂ experimental conditions. Regardless of plant type or O₂ treatment, the $\delta^{13}\text{C}_{\text{ch_substr}}$ went from ~50‰ after 6 min from the light–dark transition to ~30‰ after 30 min in the dark, while after 3 h in the dark it represented only ~10‰ [see Fig. 6; data of $\delta^{13}\text{C}_{Rd(3h)}$ approaching $\delta^{13}\text{C}_{Rd(24h)}$ are shown in Table 4]. Tcherkez *et al.* (2010) estimated on sunflower (*Helianthus annuus*) that recent assimilates provide 40–60% of the substrates for R_d (via a pool with a half-life of several hours) both in the light and in the dark. A similar contribution of recent assimilates to R_d was determined by Nogués *et al.* (2004) on French bean (*Phaseolus vulgaris*) leaves during ~2 h in the dark following illumination, which indicates that leaf respiration was fed by a mixture of recent and older substrates.

The tendency for a lower leaf $\delta^{13}\text{C}_{Rd}$ in *gdch*-KD plants compared with the WT following leaf light exposure under photorespiratory conditions may partially depend on the higher Δ_o in the *gdch*-KD plants during leaf photosynthesis in the L_{ch} at approximately current ambient $p\text{O}_2$. A greater Δ_o would cause (recent) carbon assimilates synthesized in the L_{ch} (Supplementary data Table S1) to produce more depleted respiratory substrates and a lower $\delta^{13}\text{C}_{Rd}$ in the *gdch*-KD compared with the WT. It is also possible that higher Δ_o in the *gdch*-KD compared with WT plants during growth under enriched atmospheric $p\text{CO}_2$ and current ambient $p\text{O}_2$ may have produced G_{ch} assimilates

feeding R_d over 3 h after the light–dark transition with slightly lower $\delta^{13}\text{C}$ compared with the WT.

The ¹³C fractionation during leaf dark respiration can change depending on species and environmental conditions (Ghashghaie *et al.*, 2003; Priault *et al.*, 2009; Werner *et al.*, 2009; Lehmann *et al.*, 2016). In addition, $\delta^{13}\text{C}_{Rd}$ is influenced by the isotopic signatures of respiratory substrates, from diverse non-homogeneous isotope distributions in the substrates (positional effects) and the different relative activities of decarboxylation pathways. However, decreasing $\delta^{13}\text{C}_{Rd}$ over time is mainly dependent on the origin of respiratory substrates, where CO₂ released from pyruvate decarboxylation is ¹³C enriched (compared with total organic matter) but relatively ¹³C depleted from acetyl-CoA metabolism through the TCA cycle (Tcherkez *et al.*, 2003). Under continuous darkness and constant t_{air} , it has been shown that $\delta^{13}\text{C}_{Rd}$ decreases due to a switch in respiratory substrates from carbohydrates to more ¹³C-depleted substrates such as lipids or proteins (Ghashghaie *et al.*, 2003; Tcherkez *et al.*, 2003).

The similar $\delta^{13}\text{C}_{Rd(24h)}$ in *gdch*-KD versus WT plants implies that the long-term substrates for the TCA cycle produced in the G_{ch} were ¹³C isotopically similar. This is further supported by similar leaf $\delta^{13}\text{C}_{\text{dm}}$ between the *gdch*-KD and WT plants. Interestingly, $\delta^{13}\text{C}_{Rd(24h)}$ was more depleted than $\delta^{13}\text{C}_{\text{dm}}$, in agreement with Tcherkez *et al.* (2003) who had found that CO₂ evolved in the dark by French bean leaves in a condition of carbohydrate starvation had a lower $\delta^{13}\text{C}$ than total leaf organic matter. This denotes potential changes in dark respiration substrates, such as carbohydrate oxidation producing ¹³C-enriched CO₂ and β -oxidation of fatty acids producing ¹³C-depleted CO₂ when compared with total organic matter.

Conclusions

Under photorespiratory conditions, the *gdch*-KD plants had altered ¹³C discrimination fractions in the light, with a lower Δ_f caused by a reduced f . This change in Δ_f and the lower Δ_{gm} lead to a higher Δ_o in the *gdch*-KD plants in comparison with the WT. The lower f in the *gdch*-KD plants was attributed to a greater α compared with the WT, suggesting the occurrence of alternative photorespiratory reactions in the GDC-impaired plants. In addition, the enhanced R_d in the *gdch*-KD compared with WT plants after photorespiratory leaf photosynthesis indicated that the photorespiratory disruption led to an additional buildup of metabolites in the light that were decarboxylated by the TCA cycle in the dark. The tendency for a more depleted $\delta^{13}\text{C}_{Rd}$ in the *gdch*-KD plants compared with the WT after photorespiratory leaf photosynthesis was mainly ascribed to a higher Δ_o before the light–dark transition and differences in the $\delta^{13}\text{C}$ of the substrates feeding R_d . These results indicate that an alteration in photorespiratory carbon metabolism can have a significant effect on leaf CO₂ exchange and ¹³CO₂ discrimination, both in the light and in the dark.

Supplementary data

Supplementary data are available at JXB online.

Methods S1. Estimate of the $^{13}\text{CO}_2$ composition of the growth chamber atmosphere during the light period.

Methods S2. Additional technical information on the system setup to measure online leaf atmosphere CO_2 , H_2O , and $^{13}\text{CO}_2$ exchange.

Methods S3. Estimate of the ^{13}C signature and total N content in the leaf biomass.

Methods S4. Estimate of the CO_2 compensation point in the absence of mitochondrial non-photorespiratory respiration.

Methods S5. Estimate of α , and evaluation of the sensitivity of f to Γ^* and α (shown in Fig. S1).

Methods S6. Estimate of the fractional contribution of respiratory substrates from leaf chamber and growth chamber carbon assimilates to the ^{13}C composition of dark-evolved CO_2 .

Methods S7. Editing of the ^{13}C composition of dark-evolved CO_2 for plants grown at an atmospheric ^{13}C composition of -41.6‰ .

Fig. S1 Sensitivity of the f parameter to Γ^* and α .

Fig. S2. Sensitivity of the f parameter to g_m , R_L , and e' .

Table S1. Data used to calculate the fractional contributions of leaf chamber and growth chamber carbon assimilates to ^{13}C composition of leaf dark-evolved CO_2 .

Table S2. Values of ^{18}O -based g_m .

Table S3. Statistics for the model used to fit leaf dark respiration rates.

Table S4. Significance for the model used to fit leaf dark respiration rates.

Table S5. Statistics for the model used to fit the ^{13}C composition of leaf dark respiration rates.

Table S6. Significance for the model used to fit ^{13}C composition of leaf dark respiration rates.

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Author contributions

SK, SC, H-CL, RAC, WPQ, and JMH generated the transgenic plant material; SvC, RG, RTE, GEE, and ABC planned and designed the experiments; RG performed leaf gas and isotope exchange measurements and analysis; NK performed the biochemical analysis; RG, SvC, RTE, GEE, and ABC interpreted the data; and RG, ABC, and GEE developed the manuscript.

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